

Coccidial infection does not influence preening behavior in American goldfinches

Adrian Surmacki · Geoffrey E. Hill

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Abstract Preening behavior in birds is important for the maintenance of thermoregulatory and ornamental functions of plumage. It has been repeatedly demonstrated that birds trade off time between plumage maintenance and other activities. However, the condition-dependent constraints of preening remain virtually unstudied. Here, we present the first experimental test of the hypothesis that intestinal parasite infection impairs preening activity. We studied male American goldfinches (*Spinus tristis*), a species with carotenoid-based plumage coloration. Following pre-alternate (spring) molt, we manipulated the health of males by infecting some birds with *Isospora* spp. coccidia and keeping others free of the infection. Although the goldfinches increased preening throughout the captive period, we found no significant effect of coccidial treatment on preening behavior. The effect of coccidia on plumage maintenance may be more pronounced under natural conditions where birds have limited access to food and engage in more activities that might limit time available for preening.

Keywords Feather soiling · Feather grooming · Parasites · Infection · Health

Introduction

Plumage maintenance is an important behavior among birds, accounting for about 9 % of the daily time budgeted (Cotgreave and Clayton 1994; Walther and Clayton 2005). The primary

functions of preening include: controlling ectoparasites load (Clayton et al. 2010), removing dirt and detritus (Lenouvel et al. 2009; Griggio et al. 2010), spreading preen wax over the surface of feathers (Moyer et al. 2003), and keeping feathers properly positioned. All of these activities are crucial for the maintenance of thermoregulatory function of plumage, which might be altered if the physical condition of feathers is disturbed either by soiling load, keratin-eating parasites, or disorder. Plumage maintenance can also help maintain plumage coloration. In many bird species, females use male coloration as a cue during mate choice (reviewed in Hill 2006). Ornamental plumage coloration can be compromised by soiling (e.g., Surmacki and Nowakowski 2007; Lenouvel et al. 2009; Griggio et al. 2010), and ectoparasites (Moreno-Rueda 2005) or enhanced by preen oil (e.g., López-Rull et al. 2010).

Feather maintenance is believed to be a time- and energy-consuming behavior (Cotgreave and Clayton 1994; Walther and Clayton 2005). Birds must trade off time and energy between preening and other behaviors such as foraging or scanning for predators (Redpath 1988; Cucco and Malacarne 1997; Randler 2005). Consequently, we may expect that any alteration of physiological condition may impose negative effects on preening activity. Surprisingly, this idea has been tested only once, using juvenile apapanes (*Himatione sanguinea*, Yorinks and Atkinson 2000). Individuals experimentally infected with malaria spent significantly less time preening than did healthy birds. Infected individuals also showed a reduction in activity, body mass, and fat stores (Yorinks and Atkinson 2000).

Here, we experimentally tested the hypothesis that parasite infection affects preening behavior in male American goldfinches (*Spinus tristis*), a sexually dichromatic songbird. Male goldfinches have bright-yellow carotenoid-based plumage coloration (McGraw et al. 2001) that females assess during mate choice (Johnson et al. 1993). We manipulated parasite infection status of birds by either infecting them with coccidia (*Isospora* sp.) or by curing them of coccidiosis with medicine.

A. Surmacki · G. E. Hill
Department of Biological Sciences, Auburn University,
331 Funchess Hall, Auburn, AL 36849, USA

A. Surmacki (✉)
Department of Avian Biology and Ecology, Faculty of Biology,
Adam Mickiewicz University, Umultowska 89,
61-614 Poznań, Poland
e-mail: adrian@amu.edu.pl

Coccidia are common intestinal parasite of many songbirds including house finches, *Carpodacus mexicanus* (Brawner et al. 2000). Their sporozoites feed on the epithelium of the small intestine, leading to significant impairment of absorption of various components of food (Hörak et al. 2004). To date, the effect of coccidia infection on preening has not been tested but coccidiosis is known to affect sexual display behavior in birds (e.g., Costa and Macedo 2005; Aguilar et al. 2008) and anti-predator behavior in mammals (Voříšek et al. 1998). Consequently, we predicted that infected individuals would spend less time preening.

Material and methods

General

We captured 57 wild male American goldfinches on the campus of the Auburn University in April 2010 using wire mesh traps baited with thistle seeds (see Hill (2002) for trapping method details). We registered standard biometric measurements: body mass (0.1 g), tarsus (0.01 mm), bill (0.01 mm), wing (0.5 mm), and tail length (1 mm). Additionally, we collected 10–15 breast feathers from each bird before coccidia treatment and at the end of the experiment for ectoparasite examination. We estimated the age of birds as “after second year” (ASY) or “second year” (SY) based on plumage characters (Pyle 1997). We randomly divided the birds into four groups of 13, 16, 14, and 14 birds. The proportions of the two age classes (ASY/SY) in each cage were as follows: 11/2, 14/2, 12/2, and 12/2 such that the total number of birds and proportion of ASY to SY were similar in all cages (chi-square test: $\chi^2=0.16$, $df=3$, $p=0.98$; chi-square test: $\chi^2=0.05$, $df=3$, $p=0.99$, respectively). There were no significant differences between groups of birds with regard to the linear biometric measures (see above) and body mass at the beginning of the experiment (mass: ANOVA, $df=3.53$, $F=0.43$, $p=0.74$; tarsus: ANOVA, $df=3.53$, $F=0.88$, $p=0.46$; bill: ANOVA, $df=3.53$, $F=1.33$, $p=0.28$; wing: Kruskal–Wallis ANOVA, $df=3.53$, $F=5.97$, $p=0.11$; tail: Kruskal–Wallis ANOVA, $df=3.53$, $F=3.63$, $p=0.30$). We randomly choose ten birds (five from medicated and five from infected group) on which to assess ectoparasites. We examined the collected feathers of these ten birds under dissection microscope ($\times 20$) to score potential ectoparasites (presumably feather lice). The only arthropods found of feathers were feather mites. There were no significant differences in numbers of mites between infected and medicated groups in pre-treatment samples (infected: median=1, $Q_{25-75}=0-1$; medicated: median=2, $Q_{25-75}=0-2$; Mann–Whiney test, $U=10.5$, $p=0.75$) and posttreatment samples (infected: median=0, $Q_{25-75}=0-0$; medicated: median=0, $Q_{25-75}=0-1$; Mann–Whiney test, $U=10.0$, $p=0.68$). We housed each group in

outdoor cages (3.7×1.5×2.4 m) with wire mesh walls and ceilings and concrete floors. Approximately one half of the ceiling was covered with a sheet of plywood to provide shade and shelter from weather. Each cage had five wooden stick perches for a total length of 5.5 m. We individually marked birds with numbered aluminum bands and a combination of two color bands. We fed birds with sunflower seed chips, thistle seeds, and water ad libitum. Until the beginning of the experiment in July (see below), all birds were provided with sulfadimethoxine, an antibiotic known to protect captive wild birds against coccidial infection (Brawner et al. 2000). Moreover, throughout the experiments, all birds were maintained continuously on the antibiotic drug tylosin tartrate (0.3 mg/ml). This drug suppresses the lethal bacterium *Mycoplasma gallisepticum* (Nolan et al. 2000) and prevents proliferation of this bacterium in captive flocks. At the beginning of the experiment, all males had completed their spring pre-alternate molt-growing bright-yellow body plumage.

Experiment design

We randomly assigned cages of birds to medicated (two cages) or infected (two cages) treatment groups. Birds in both groups were treated with sulfadimethoxine since they were caught (April) until beginning of experiment (July 13) to exclude effects of coccidia during the period when they were adjusting to captivity. Birds in the infected group were taken off of sulfadimethoxine treatment and infected with coccidian by addition of *Isospora* sp. oocysts to drinking water (see below for details). Thus, we created two distinct groups of birds: medicated with no detectable coccidia infection and infected in which the prevalence of coccidia was 100 % (see next section for details). The experiment started on July 13 (day “0”), when coccidiostatic medicine was terminated in treatment birds and lasted 33 days. Between the eighth and third days preceding the start of experiment, we scored grooming behavior of birds (5 days in total). The second score of grooming behavior took place on 34th and 37th day of experiment (4 days in total). We failed to quantify preening behavior for four males; therefore, the total sample size used in analysis was 53.

Earlier studies indicated that sulfadimethoxine suppresses, but does not eliminate, isosporan coccidiosis (Brawner et al. 2000). Consequently, several days after the treatment with sulfa drugs stopped, birds should begin to pass oocysts (Brawner et al. 2000). We randomly collected feces from the floors of cages of treatment groups on the 10th and 14th days of the experiment and found oocysts present in only 50 % of feces tested. Therefore, on days 15th and 16th of the experiment, we infected drinking water with powdered feces collected from wild birds (0.014 g of per 100 ml). Feces were collected during the afternoon when the coccidia excretion is the highest (Brawner and Hill 1999). This method mimics real

infection in the wild. Coccidian of the genus *Isospora* is transferred from infected to uninfected birds by coccidian oocysts passing in feces that are then ingested by foraging birds (Box 1981).

The coccidia manipulation protocol may have resulted in ~50 % of birds (see above), being exposed to infection for 33 days while the rest infected for 18 days. Despite this divergence, we are confident that our experiment design allowed enough time for changes in grooming to be visible. In Dolnik and Hoi's (2010) study, House Sparrows showed changes in aggressive behavior 9–10 days after reinfection with coccidia. In another study on Eurasian Blackbirds, the peak number of oocysts in feces occurred on tenth day after infection, and then declined (Filipiak et al. 2009).

Coccidian infection analysis

We collected feces from all birds on 9 and 11 August (days 27th and 29th of the experiment), between 1700 and 2000 hours. For fecal collection, we temporarily housed goldfinches individually in small (0.5×0.5×0.5 m) cages with mesh bottoms between. We placed a steel pan lined with wet paper towels beneath each cage to collect feces. We then moved feces to 5-ml vials containing 2 ml of 2.2 % potassium dichromate and ground them with a wooden dowel. Vials remained open for 3 days to allow oocysts to sporulate. Then, vials were tightly closed and refrigerated until oocyst' counting. We measured the mass (to the nearest 0.001 g) of each vial containing potassium dichromate immediately before and after feces were added to the vials. This procedure allowed us to determine the mass of fresh feces as the difference between masses of empty and feces containing vials.

We scored coccidian oocysts using a hemocytometer under ×100 magnification by counting all oocysts visible within a grid of nine 1×1 mm². For each bird sample, we averaged four counts of oocysts. Before each count was made, the vial was vigorously shaken to mix the contents. To calculate the total number of oocysts within 1 g of feces, we extrapolated average score from hemocytometer grid area volume (900 nl) to the whole sample volume (2 ml). The number of oocysts per 1 g of feces was positively correlated with mean oocyst number per feces sample ($r_s=0.82$, $p<0.01$, $n=19$) but not with feces mass ($r_s=-0.40$, $p=0.09$, $n=19$). Because precise calculation of the number oocyst per 1 g of feces was not possible in all cases (see “Results” for more details), in subsequent analyses, we used the number of oocyst per feces sample as a measure of coccidia abundance.

The coccidia oocysts were distinguished as *Isospora* spp. by Dr. Christine A. Sundermann (Auburn University) under a microscope (×1,000).

Preening behavior

We used a protocol modified from Lenouvel et al. (2009) to quantify grooming behavior. We sampled the behavior of males in random order 60 times for 10 s intervals. Half of the behavioral samples ($n=30$) were gathered in the morning (0730–1100 hours) and the other half ($n=30$) in the afternoon (1400–1830 hours). A.S. observed birds through small two-way mirrors mounted in the wall of building adjacent to cages and recorded the following grooming behaviors: preening (the bird takes a feather in its beak), ruffling (the bird shakes entire plumage), and scratching (the bird scratches a part of its plumage with a foot). The maximum score that a male could get for a particular grooming behavior was 60 (grooming observed in all 10 s samples).

Statistical analysis

We tested the normality of the data distribution using Shapiro–Wilk tests. Preening scores were log₁₀ transformed to obtain a normal distribution (raw data: Shapiro–Wilk test, $W=0.95$, $p=0.02$; transformed data: Shapiro–Wilk test, $W=0.97$, $p=0.17$). Changes in preening activity were analyzed using General Linear Mixed Models (GLMM) with Measurement (preening at pre- and posttreatment phases) and Treatment (medicated or infected) as fixed factors. To test whether changes in preening depended on infection level, Measurement×Treatment interaction was included in the model. Because both medicated and infected birds were kept in two cages, we included cage identity as a random factor.

We performed a priori power test to assess sample size needed to confidently test the hypothesis about the effect coccidia infection on changes in preening activity (Measurement × Treatment interaction). We used G*Power 3.1.7 software (Faul et al. 2007) with the following settings: effect size=0.25, $\alpha=0.05$, nonsphericity correction $\varepsilon=1$. The sample size needed to have an 80 % chance of detecting a difference if such a difference existed is 34, which is below the sample size used in the study ($n=53$).

Results

We found coccidian oocysts in all fecal samples collected from infected males at the end of the experiment. Median number of oocysts per 1 g of fresh feces was 14×10^5 ($Q_{25-75\%}=0.97\times 10^5-34\times 10^5$, $n=19$). For eight birds, the mass of feces was too low to measure, nevertheless, even those samples contained oocysts. Therefore, we also calculated the mean oocyst number per feces sample (see “Material and methods”). Median oocyst number per feces sample for all infected males was 44 ($Q_{25-75\%}=2.50-93.00$, $n=27$). Zero

Table 1 Results of GLMM analysis of the effect of experimental infection with *Isospora* coccidia on the American goldfinches preening behavior

Variable	F	N	D	df	P	Estimate±SE	Wald Z	P
Measurement	8.95	1	100.47		<0.01			
Treatment	0.68	1	0.68		0.53			
Measurement× Treatment	0.06	1	100.47		0.81			
Cage						0.003±0.004	0.69	0.49

Measurement of the same individual (preening at pre- and posttreatment phases) and Treatment (medicated or infected) were entered as fixed factors and Cage as a random factor

oocysts were found in samples collected from medicated birds.

Birds increased preening intensity at the end of the experiment (Table 1, Fig. 1). The magnitude of change in preening behavior did not differ between treatment groups, and variation in preening activity was not related to cage identity (Fig. 1, Table 1).

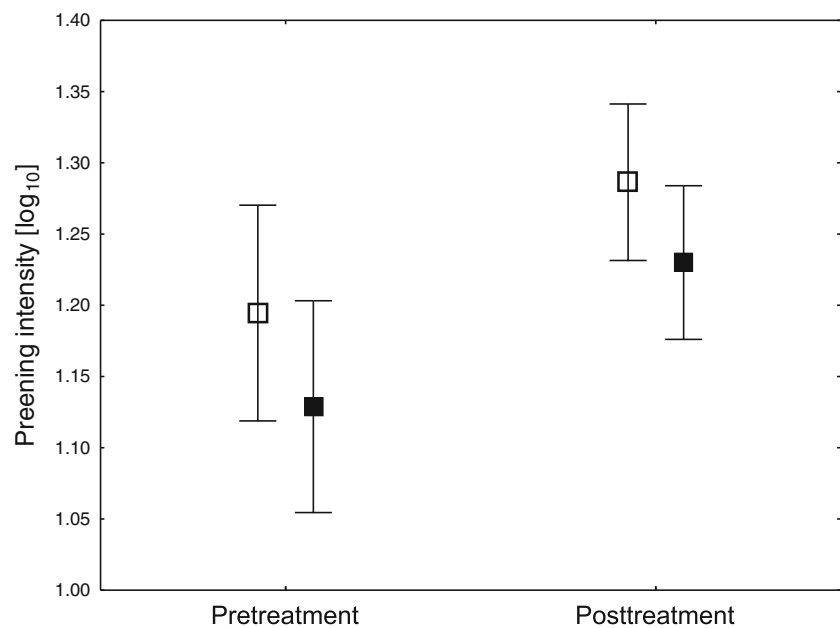
Discussion

We predicted that goldfinches infected with *Isospora* coccidia would devote less time to plumage maintenance. The results of our experiment did not support our prediction. In the course of the study, all of the goldfinches increased preening intensity but infection had no significant effect on preening.

Fecal analysis confirmed that we succeeded in creating two distinct groups: healthy birds that did not shed oocysts (prevalence 0 %) and infected birds with 100 % prevalence of coccidia. According to a previous study on a related species (Brawner and Hill 1999), mean level of infection in our birds should be regarded as massive. Coccidiosis is a serious, chronic intestinal infection of songbirds (Duszynski et al. 2000). Hōrak et al. (2004) found that coccidia significantly depressed serum albumin, triglycerides, and fat-soluble antioxidants (carotenoids and vitamin E) in closely related greenfinches (*Carduelis chloris*). In captivity, American goldfinches are very sensitive to coccidiosis (McGraw and Middleton 2009). Wild birds kept in aviaries without coccidiostatic medicines often die of coccidiosis within few weeks (G. E. Hill not published observation, McGraw and Hill 2000). Male American goldfinches that have coccidial infection during molt grow yellow breast feathers that are less bright than males that are not infected (McGraw and Hill 2000).

Our prediction that coccidial infection would affect feather maintenance behavior was based on previous surveys suggesting that coccidia influence the activity level of birds. Correlational and experimental studies on blue-black grassquits (*Volatina jacarina*) demonstrate that coccidial infection negatively influences the frequency of courtship leaps (Costa and Macedo 2005; Aguilar et al. 2008). House Sparrows (*Passer domesticus*) infected with *Isospora michaelbakeri* tend to experience a lower hierarchy position in captive flocks (Dolnik and Hoi 2010). The behaviors related to coccidial infection in these previous studies, however, did not necessarily compromise the survival of the infected bird. It seems likely that when they become ill, individuals

Fig. 1 Mean ($\pm 95\%$ CL) preening activity at preening at pre- and posttreatment phases. Open squares are medicated and filled squares infected males



prioritize those physiological processes and behaviors that are needed to help them cope with the parasite and recover. Maintaining plumage in good condition seems likely to be one of the prioritized behaviors. Moreover, social behavior and sexual display are probably more energetically demanding than preening, which makes them more sensitive to coccidiosis.

Another possibility is that captive conditions buffered the effects of infection on preening behavior. Unlike wild goldfinches, birds used in this experiment did not have to trade off time between plumage maintenance and other vital activities like predator avoidance, food searching, courtship, or parental care. The results of our study do not exclude the possibility that coccidia infection diminishes preening behavior when resources or time are limiting. Future research should be performed in more natural conditions in which the effects of coccidia on the preening behavior might be stronger.

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